ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Total phenolic content, flavonoid concentration and antioxidant activity of *Chrozophora Plicata* leaves and seeds extracts

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Manuscript Info

Manuscript History:

Received: 16 June 2015
Final Accepted: 15 July 2015
Published Online: August 2015

Key words:

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Abstract

The herbal medicines derived from various plants extracts are being used for thousands of years to flavor and conserve food and utilized to treat a wide variety of clinical diseases. Leaves and seeds *Chrozophora plicata* (*Euphobiaceae* family) are distributed in West African and Asia is selected for the study to investigate its anti-oxidative activities of different solvents in polarity increase order were used to extract *Chrozophora plicata* leaves and seeds. The ethyl acetate and methanol extract of the leaves part have the highest total reducing power activity. While on the seeds part the ethyl acetate extract have more potent free radical scavenging activity than all the other extracts using DPPH free radical scavenge capacity assay the petroleum ether extract of the leaves and seeds part has less antioxidant activity; IC_{50} was calculated and compared with propyl gallate as standard. Phytochemical screening of the two parts indicates the presence of alkaloids, coumarins, flavonoids, sterols, saponins and tannins with different concentration. Quantitative analysis of the two parts of *Chrozophora Plicata* for phenolic, flavonoids and tannins compounds revealed that the total phenolic content ranged from 10.625 to 6107 and 21.625 to 2878.125 mg/L of dry weight of leaves and seeds extracts respectively which expressed as gallic acid equivalents. The total flavanoids content of extracts determined by Aluminum chloride colorimetric assay ranged from the 364 to 1697 and 25.28571 to 981.3571 mg/L of dry weight of leaves and seeds extracts respectively expressed as quercetin equivalents. The total tannins concentrations varied from 64.57143 to 2533.213 mg/l and 62.5714 to 28571 mg/l for leaves and seeds respectively. The present study demonstrated that *Chrozophora Plicata* leaves and seeds have potent antioxidant activity with the presence of effective phytochemical compounds.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, many of these isolations were based on the uses of the agents in traditional medicine (Cragg and Newman 2001).

A total of 122 biologically active compounds have been identified, derived only from 94 species of plants. A conservative estimate of the number of flowering plants occurring on the planet is 2, 50,000. Of these, only about 6% have been screened for biological activity and a reported 15% have been evaluated phytochemically. Consistent

findings should be carried out to discover a probable abundance of medicinal extracts in these plants (Turker and Usta 2008).

Herbs are natural products and their chemical composition varies depending on several factors, such as botanical species, used chemotypes, the anatomical part of the plant used (seed, flower, root, leaf, fruit rind, etc.), also storage, sun, humidity, type of ground, time of harvest, geographic area etc. This variability can result in significant differences in pharmacological activity: involving both pharmacodynamics and phamacokinetics issues (Park 2008).

Herbal medicines are an essential and growing part of the international pharmacopeia. Knowledge of their medicinal properties is growing as a result of research and testing, which will make them an increasingly safe alternative or a preferred option to allopathic medicine. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse sideeffects (Parekh and Chanda 2006).

In fact, most of the major anticancer drugs are derived from plants or microorganisms. Important examples include bleomycin, doxorubicin, daunorubicin, vincristine, vinblastine, mitomycin, streptozocin, and most recently, paclitaxel, ironotecan (a camptothecin derivative), and etoposide and tenoposide (podophyllotoxin derivatives) (Ebadi, 2007).

Phenolic acids are large and heterogeneous group of biologically active non-nutrients. They are present in plants as hydroxylated derivatives of benzoic and cinnamic acids (Shahidi and Naczk, 1995). Phenolic compounds are important in the defense mechanisms of plants under different environmental stress conditions such as wounding, infection, and excessive light or UV irradiation (Dixon and Paiva, 1995). Phenolics are not only unsavory or poisonous, but also of possible pharmacological value (Strack, 1997).

Euphorbiaceae, the spurge family, is a large family of flowering plants with 300 genera and around 7,500 species. Most spurges are herbs, but some, especially in the tropics, are shrubs or trees (Gibbs RD. 1974).*Chrozophora* genus is a plant of the family *Euphorbiaceae* and the sole genus comprised in the subtribe chrozophorinae. It comprises 8-7 species, which are mostly monoecious herbs under shrubs. This genus is distributed in Pakistan, India, and West Africa and Previous phytochemical investigation of the genus *Chrozophora* resulted in the isolation of several types of chemical constituents including essential oils, terpens, sterols, phenylpropanoid glycosides, xanthones, chromone and flavonoids. It was reported that the plant contained essential oils and flavonoids.

C.Plicata Monoecious, annual to perennial herb up to 50 cm tall; stem angular, much-branched from the base, densely hairy with stellate hairs, yellowish or pinkish. In Sudan pounded stems or whole plants are applied to wounds to improve healing. In Ethiopia an infusion of the seeds and leaves is taken as a laxative. The plant is also used medicinally in Saudi Arabia, Pakistan and India, e.g. against jaundice and to purify blood.

In Senegal the plant is not browsed by most stock, except occasionally by sheep and goats, as it causes vomiting and diarrhea. In Kenya camels graze it. The fruits yield a purplish blue dye, which is used in east Africa to dye mats.

Scientific reports indicate that the ethanol leave extract of the plant has in-vitro free radical scavenging activity, also the leaves of *Chrozophora plicata* plant contain triterpenoids and related compounds (sterols, alcohols and hydrocarbons), phenolic compounds (flavonoids, lignans, coumarins, tannins, phenanthrenes, quinones, phenolic acids, etc.) that are possessing antioxidant properties (kumar *et al*., 2013), *Chrozophora plicata* possesses ulcer protective principles and flavonoids may be responsible for gastroprotective activity. (Kadiri and Avanapu., 2014)..

new secondary metabolites have been isolated from *Chrozophora plicata* β-sitosterol, methyl p-coumarate , 4-hydroxyphenylacetic acid, succinic acid, speranberculatine A ,β-sitosterol-3-O-β-D-glucopyranoside and apigenin-5-O-β-D-glucopyranoside have also been isolated (Naheed Riaz *et al*., 2013).

The main aims of this study are to evaluate antioxidantcapacity (total phenolic content and free radical scavenging ability), and to screen for phytochemicals content in seeds and leaves of *Chrozophora Plicata medicinal plant from Leguminosae family.*

MATERIALS AND METHODS

Chemicals and reagents:

Gallic acid, tannic acid, Querstin,1,1-diphenyl-2-picrylhydrazyl radical (DPPH), sodium hydroxide ,sodium nitrite ,ferric chloride ,potassioum ferrous cyanide ,sodium bicarbonate ,aluminum chloride and Folin Ciocalteau reagent were bought from Sigma-Aldrich, USA

Collection of the plant material

The fresh plant of *Chrozophora Plicata* was collected from Khartoum, Nile street. The plant was identified and was authenticated by the herbarium unit of National Research Institute, The plant material was cleaned, separated into leaves, seeds and pods and they were shade dried. When the plant materials were thoroughly dried, they were

coarsely powdered using a Manuel grinder. The powder was stored in an air tight, light resistant container for further analysis.

Preparation of the plant extracts

Forty grams, thirty grams of the fine powdered plant leaves, seeds respectively were separately defatted with petroleum ether (60-80)^{o}C. The defatted materials were sequentially extracted with chloroform, ethyl acetate and methanol by soxhelt extractor in 200 mL of the relevant solvent, After filtration through Whatman filter paper (No. 1), respective solvents were evaporated under reduced pressure using a rotary evaporator (Buchi rotavapor II) at 40^{o}C to obtain the extracts. The extract in each case was weighed, transferred to small container and stored in a refrigerator at 4^{o}C until tested.

Qualitatative Tests for secondary metabolites

Phytochemical screening of the prepared extracts

The prepared extracts were tested for their presence or absence of alkaloids, saponins, cardiac glycosides, flavonoids, sterols and triterpenes, sesquiterpene lactons, tannins and sugars according to methods described by Harborne, (1984) and Sofowora, (1993)

Quantitative determination of total phenols, flavonoids and tannins contents in *C. Plicata* leaves and seeds extracts:

1- Total Phenolics Content:

The total phenolic content of each extract was determined by adopting the method as described by wolfe *et al* (2003). The total phenolic contents were expressed as gallic acid equivalents (mg/l) using the following equation based on the calibration curve: y=0.001x+0.136 where x = concentration of gallic acid (mg/l) corresponding to y the absorbance with R=0.995. A calibration curve was prepared using gallic acid (100-800 mg/l) as standard and used for calculation of total phenolic compounds.

2- Total flavonoids content:

The total flavonoids content was determined by adopting the method described by Shanukha *et al* (2012). Absorbance was measured at 415 nm against a reagent blank. Using Shimadzu model 1800 double beam spectrophotometer. Total flavonoids content was expressed as quercetin (mg/l) using the following equation based on the calibration curve Y=0.000x +0.064, where y was the absorbance and R= 0.997, calibration curve was constructed, using quercetin (50-700 mg/l) as standard and total flavonoids content of the extracts (mg/l) expressed as quercetin equivalents.

3- Total tannins content:

The tannins content was determined by using $FeCl_3$ and gelatin test (Shivakumar *et al*., 2012). Absorbance was measured at 510 nm against a reagent blank using Shimadzu model 1800 double beam spectrophotometer. The total tannins content was calculated using the following equation y=0.001x+0.066, where x=concentration of tannic acid (mg/l) corresponding to optical density. A calibration curve was constructed, using tannic acid (100-800 mg/l) as standard with R= 0.9936 and total tannins content of the extracts (mg/l) expressed as tannic acid equivalents.

Determination of antioxidant activity

DPPH radical scavening assay

The DPPH radical scavenging was determined according to the method of Shimada *et al* (1992). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

IC_{50} calculation:

The IC_{50} (the concentration of test ,material, which possess 50% inhibition of free radicals) of all the extracts and their fractions was determined by monitoring the effect of different concentrations ranging from 500-62.25 µg/ml. the IC_{50} of the extracts and their fractions were calculated using EZ-FIT Enzyme kinetic program (Perrella scientific, U.S.A).

Results and discussion

Quantity of extracts

Successive extraction of leaves of *Chrozophora Plicata* gave the highest yield with methanol followed by Chloroform, petroleum ether and finally ethyl acetate: 16.52; 1.7; 1.4 and 0.89 % respectively. Regard to seeds, the

highest yield was observed with methanol followed by ethyl acetate, chloroform and finally petroleum ether 4.42; 4.9; ; 5.2, 5.7% respectively figure (1).

Fig (1): extractive yields of *C. Plicata* seeds and leaves

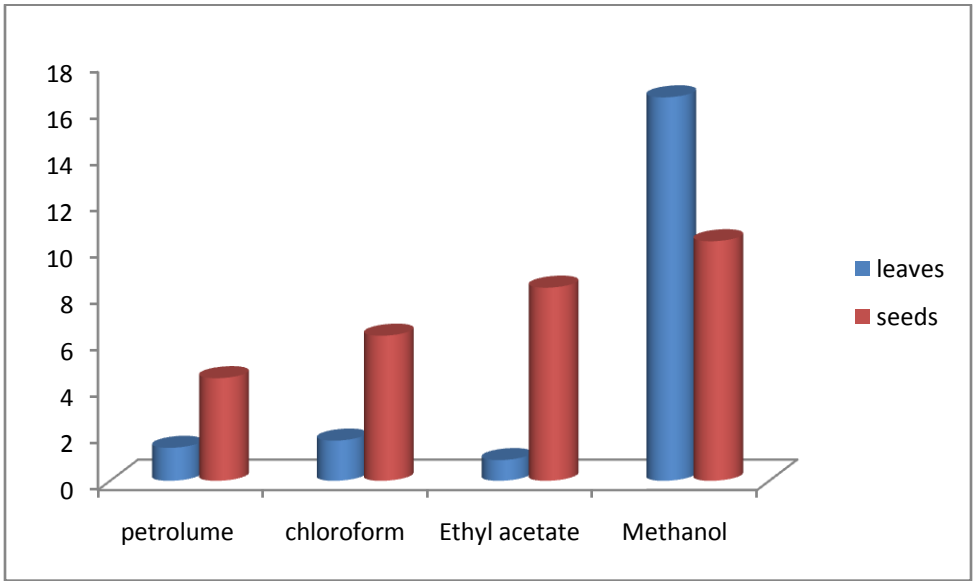


Table 1: Preliminary phytochemical screening of leaves extracts of *Chrozophora Plicata*

Class of compound	Test Reagent	Extracts			
		PE	**$CHCl_3$**	**EtOAc**	**MEOH**
Alkaloids	Wagner	-ev	+++	++	-ev
	Mayer	-ev	+++	++	+
	Dragendrorff	-ev	++	++	+
Flavonoids	Lead acetate	++ +	++ +	++ +	+++
	$Fecl_3$	++	++ +	+ ++	+++
	KOH 1%	+	+	+++	++ +
	$ALCL_3$	++	++	+++	++
Sterols	Salkowski	+ +	+ +	++	-ev
	Liebermann	+ ++	+ +	+ +	-ev
Triterpenes	Salkowski	-ve	-ve	-ve	-ve
	Liebermann	-ve	-ve	-ve	-ve
Glycosides	conc. H_2SO_4	+++	-ev	+++	+++
	relleK	+++	-ev	+++	+++
Tannins	$FeCl_3$	-ev	-ev	++	-ev
	Lead acetate	-ev	-ev	++ +	+++
	Gelatin	-ev	-ev	+++	++
Lignin	Labat	-ev	+ +	-ev	++
Saponin (powder of leaves)		+ +			
Coumarin (powder of leaves)		+ +			

Note: "+" low, "++" average, "+++" high, "-" Not detected

PE: Petroleum Ether

Table 2: Preliminary phytochemical screening of Seeds extracts of *Chrozophora Plicata*

Class of compound	Test Reagent	Extracts			
		PE	**$CHCl_3$**	**EtOAc**	**MEOH**
Alkaloids	Wagner	++	+ +	-ev	-ev
	Mayer	-ev	+ +	+ +	-ev
	Dragendrorff	+++	+++	-ev	-ev
Flavonoids	Lead acetate	+++	++ +	+ ++	-ev
	KOH 1%	++	+ +	++	-ev
	$FeCL_3$	-ev	-ev	++	-ev
Sterols	Salkowski	++	+ +	-ev	-ev
	Lieberman	+++	+ + +	-ev	-ev
Triterpenes	Salkowski	-ev	-ev	-ev	+++
	Liebermann	-ev	-ev	+ + +	-ev
Tannins	$FeCl_3$	-ev	-ev	++	-ev
	Lead acetate	-ev	-ev	++ +	+++
	Gelatin	-ev	-ev	+++	++
Lignin	Labat	-	+ + +	+ +	+++
Saponin (powder of seeds)	+				
Coumarin (powder of seeds)	+ +				

Note : "+" low , "++" average , "+++" high , "-" Not detected

Phytochemical screening of *Chrozophora Plicata* leaves extracts indicate the presence of flavonoids, alkaloids and tannins in methanol and ethyl acetate of leaves extract in high concentration while the tannins and flavonoids don't found in petroleum ether and chloroform extracts showed in Table (1). Whereas, analysis of seeds extracts of *Chrozophora Plicata* indicates the presence of flavonoids in all seeds extracts while tannins and Triterpeins don't found in petroleum ether and chloroform extract also the sterols and alkaloids don't found in methanol and ethyl acetate extracts as showed in Table (2).

From the preliminary previous studies, it was found that the leaves of *Chrozophora Plicata* plant contain Triterpenes and related compounds (sterols, alcohols and hydrocarbons), phenolic compounds (flavonoids, lignans, coumarins, tannins, phenanthrenes, quinones, phenolic acids, etc.) (Kadiri and Avanapu.,2014).

Quantitative analysis for total phenols, flavonoids and tannins content in leaves and seeds extracts of *Chrozophora Plicata:*

The total phenolic, flavonoid and tannin contents of leaves and seeds extracts of *Chrozophora Plicata* were evaluated and results are presented in Table (3). The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.0008x + 0.0397$, ($R2 = 0.999$). The values obtained for the concentration of total phenols are expressed as mg of GA/l of extract. The total phenolic contents in the examined leaves extracts ranged from 10.625 to 6107 mg GA/l. The highest concentration of phenols was measured in methanolic, ethyl acetate, chloroform and petroleum ether extracts in the leaves. The total phenolic content in the seeds was ranged from 21.625 to 2878.125 mg GA/l. The highest concentration of phenols was measured in methanolic, ethyl acetate, petroleum ether and chloroform extracts in the seeds part. The total phenolic content in plant extracts of the species *M. peregrinum* depends on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Mohsen and Ammar, 2008; Zhou and YU, 2004).

The concentration of flavonoids in various plant extracts of the *Chrozophora Plicata* was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalent. The standard curve equation that used in calculation was $y = 0.0007x + 0.0537$. The concentration of flavonoids in plant extracts of *Chrozophora Plicata* leaves part ranged from 364 to 1697 mg/L. Ethyl acetate extract contains the highest flavonoid concentration whereas The lowest flavonoid concentration was measured in chloroform extract, the concentration of flavonoids in plant extracts depends on the polarity of solvents used in the

extract preparation (Min and Chun-Zhao, 2005). The concentration of flavonoids in seeds part ranged from 25.28571 to 981.3571 mg/l also the highest concentration of flavonoids was found in ethyl acetate, methanol, chloroform and petroleum ether extracts, respectively.

Tannin content was calculated as tannic acid equivalent (the standard curve equation: Y= 0.0028X+.0824 and values ranged between 64.57143 to 2533.213 mg/l for leaves part. The highest tannin content (2533.213 mg/l) was observed in methanol followed by ethyl acetate (1826.665mg/l), petroleum ether extracts (137.23 mg/l) and chloroform (64.57143 mg/l) extracts respectively. On the other part (seeds) the value ranged from 62.5714 to 28571 mg/l. The highest tannin content (28571 mg/l) was observed in ethyl acetate followed by methanol (1811.375 mg/l), chloroform (246.83999 mg/l) and petroleum ether extracts (62.5714 mg/l), respectively.

Table (3):

Concentration of total polyphenols, flavonoids and total tannins in the plants extracts:

Phenolic	Part of plant	Petroleum ether Mean	$CHCL_3$ Mean	Ethyl acetate Mean	Methanol Mean
Total polyphenol	Leaves	10.625	745.937	1601.25	6107
	Seeds	296.4313	21.625	2233.25	2878.125
	P-value	0.112	0.001	0.091	0,012
Total tannins	Leaves	137.23	64.57143	1826.665	2533.213
	Seeds	62.5714	246.839	99.28571	1811.375
	P-value	0.042	0.000	0.009	0.009
Total flavonoids	Leaves	416.3571	364	1697	588.6429
	Seeds	25.28571	85.07143	981.3571	717.4286
	P-value	0.070	0.034	0.002	0.187

Total polyphenol is expressed as mg Gallic acid/g of dry plant material. Total Flavonoids is expressed as mg quercetin/g of dry plant material. Total tannin is expressed as mg of tannic acid/g of dry plant material.* significantly different from the other at P< 0.05.

Statistical analysis:

Data are presented as the mean ± SD of triplicates determination. Data were analyzed by SPSS statistical software (version 16). Values were considered significantly different at $p < 0.05$. .

Fig 2: Total phenols, flavonoids and tannins contents in leaves extracts of *Chrozophora Plicata*

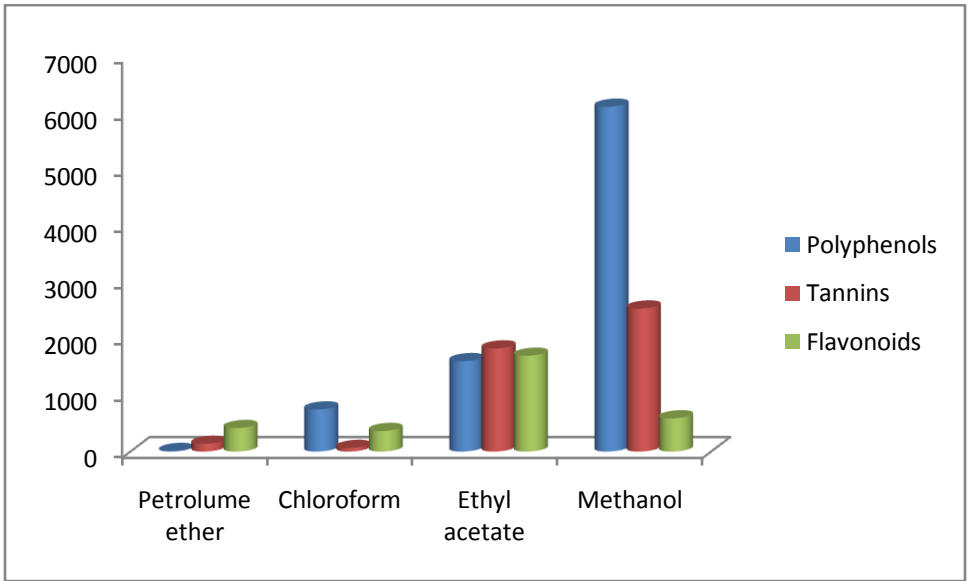
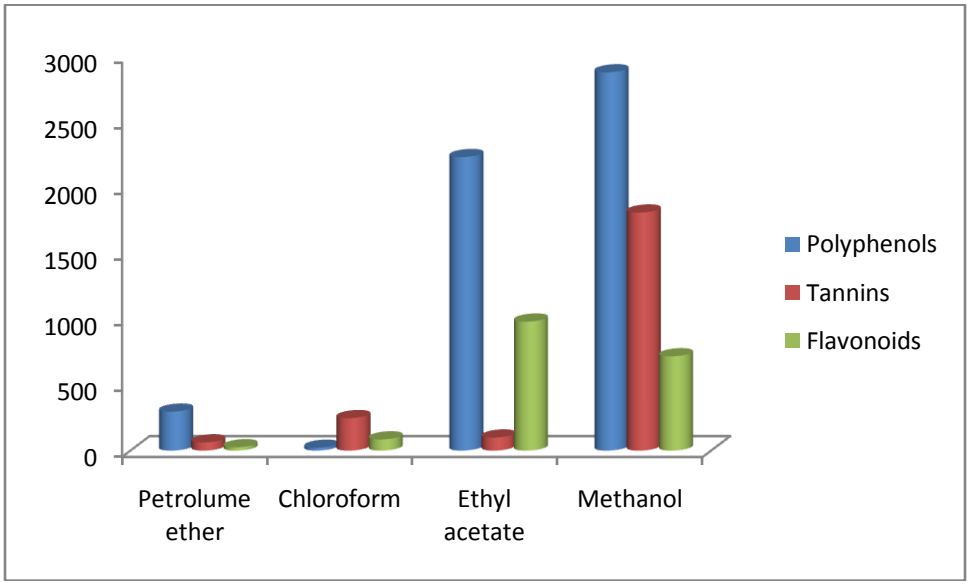


Fig 3: Total phenols, flavonoids and tannins contents in seeds extracts of *Chrozophora Plicata*



Antioxidant activity:

The antioxidant activity of different plant extracts from *C. Plicata* was determined using a methanol solution of DPPH reagent. DPPH is very stable free radical. Unlike *in vitro* generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when antioxidant molecule quench DPPH free radicals (i.e. by providing atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colourless- /bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band (Amarowicz *et al*., 2003). The antioxidant activity of four different extracts of *C.Plicata* is expressed in terms of percentage of inhibition (%) and IC_{50} values (µg/ml) table (4). The standard values were obtained and compared to the values of the antioxidant activity. The standard substance that used in the test was propyl gallate. In-vitro antioxidant activity of the petroleum ether, chloroform, ethyl acetate and methanol extracts from leaves and seeds of *C. Plicata* was evaluated using DPPH assays table (3). The ethyl acetate and methanol of leaves extracts showed the highest activity 97% and 85% the IC_{50} value was found to be (0.078 – 0.062 µg/ml) respectively while the ethyl acetate and methanol of seeds part showed also high inhibition activity 79 % and 67% with IC_{50} 0.239-0.119 µg/ml respectively but the petroleum ether and chloroform extracts of the seeds part were inactive of DPPH scavenging activity. The chloroform leaves extract showed low DPPH scavenging activity with inhibition percentage 44%. Due to moderate and low activity of petroleum ether and chloroform extract of the leaves and seeds, IC_{50} was not calculated. The high DPPH radical scavenging activities of the various solvent extracts which are comparable to standard antioxidants used suggest that the extracts have compounds with high proton donating ability and could serve as free radical inhibitors. However, the organic solvent extract from the leaves and seeds demonstrated a more remarkable anti-radical activity. Antioxidant contents of *C. Plicata* were previously reported by (kumar K.Sunil, *et al.,* 2013)

Table 4: Antioxidant activity of seeds – leaves extracts of *Chrozophora Plicata*.

Extract	%RSA±SD (DPPH) (leaves)	IC50 (µg/mL) (Leaves)	%RSA±SD (DPPH) (seeds)	IC50 (µg/mL) (seeds)
Petroleum ether	13 ± 0.12	-	inactive	-
Chloroform	44 ± 0.32	-	9 ± 0.26	-
Ethyl acetate	97 ± 0.02	0.078 ± 0.00	79 ± 0.14	0.239 ± 0.00
Methanol	95 ± 0.02	0.062± 0.03	67 ± 0.14	0.119 ± 0.01
Propyl gallate	84 ± 0.02	0.055 ± 0.00	84 ± 0.02	0.055 ± 0.00

Conclusion

In conclusion, it could be stated that the ability of free radical scavenging activity of leaf and seed parts of *Chrozophora Plicata* might be due to the presence of antioxidants and secondary metabolites both of which could serve as free radical inhibitors or scavenger. The present study revealed that the leaves and seeds of *Chrozophora Plicata* appear to be good sources of essential antioxidants by their radical scavenging activity which can have novel therapeutic value against various degenerative diseases.

References

Cragg GM, Newman DJ. (2001). Medicinals for the Millennia. Annals of the New York Academy of Sciences 953: 3-25.

Dixon R. A., Paiva N. L. (1995). Stress-induced phenylpropanoidmetabolism. *Plant Cell*, 7, 1085–1097
.
Gibbs RD. (1974). Chemotaxonomy of flowering plants, McGill- Queen's University press, Montreal, Canada, Prota. *Medicinal plants/Plantes médicinales*, 11(1), 124-128.

Harbone, J. B (1984). Phytochemical Methods A. Guide to Modern Techniques of Plant analysis Chapman and Hill, London, pp 182-20.

Harborne J. B. (1994). The Flavonoids: Advances in Research since 1986. London, UK: Chapman & Hal.

Kadiri.Sunil kumar, A.Srinivasa Rao, K.Satyanarayana. (2013). Evaluation of in vitro antioxidant activity of plant *Chrozophora plicata , BioMedRx, ,1(1),47-50*.

Kadiri Sunil kumar, Avanapu Srinivasa Rao .2013. evaluation of antiulcer activity of plant *Chrozophora Plicata*, *Int J Pharm*, 3(4): 774-778.

Naheed Riaz, Asia Tabussum, Muhammad Saleem, Muhammad Ashraf, Romana Nasar , Bushra Jabeen, Abdu Malik and Abdul Jabbar, (2013), New lipoxygenase inhibitory sphingolipids from *Chrozophora plicata*. Journal of Asian Natural Products Research Volume 15, Issue 10.

Parekh J, Chanda S. (2006). *In vitro* antimicrobial activities of extracts of *Launaea procumbens* Roxb. (*Labiateae*), *Vitis vinifera* L. (*Vitaceae*) and *Cyperes rotundus* L. (Cyperaceae). *African Journal of Biomedical Research* 9: 89-93

Park JH. (2008) Evidence-based herbal medicine in efficacy and safety assessments. Oriental Pharmacy and Experimental Medicine 8: 103-110.

Shahidi F., Naczk M. (1995). Food Phenolics, Sources, Chemistry, Effects, Applications.Lancaster, USA: Technomic Publishing Company, Inc.

Shanmukha,BAI.,Patel,J.and Settee,R.S. (2012).spectroscopic determination of total phenolic and flavonoids contents of *Sesbania Grandiflora* (Linn) Flower. *Am.Jpharm.Tech.Res*.2(2):309-405.

Shimada K, Fujikawa K, Nakamura T. (1992). Antioxidatative properties of xanthan on the antioxidation of *Soybean* oil in cyclodextrin emulsion. *J Agric Food Chem*; 40:945-8.

Shivakumar, B.S., Ramaiah,M.,Hema M.,Vijay Kumar, M. and Vaidya,V.P.(2012) .Quantitative determination of total content of phenol, flavonoid and tannin in leaf extract of *Barlaria Buxifolia* L.*Am. J. pharm Tech Res*.2 (5):418-422.

Sofowora A. (1993). Medicinal plants and traditional medicine in Africa.Chichester John,Wiley & Sons New York. p. 256.

Strack D.(1997). Phenolic metabolism. In: Dey P. M., Harborne J. B., eds. *Plant* Biochemistry. London, UK: Academic Press, 387–416.

Turker AU, Usta C. (2008). Biological screening of some Turkish medicinal plant extracts for antimicrobial and toxicity activities. *Natural Product* Research 22:136-146

.

Wallace G., Fry S.C. (1994). Phenolic components of the plant cell wall. *Int. Rev. Cytol*., 151, 229–267.

Wolfe, K., Wu, X., and Liu, R.H. (2003). Antioxidant activity of apple peels. *Journal of Agriculture* and Food Chemistry 51: 609- 614.